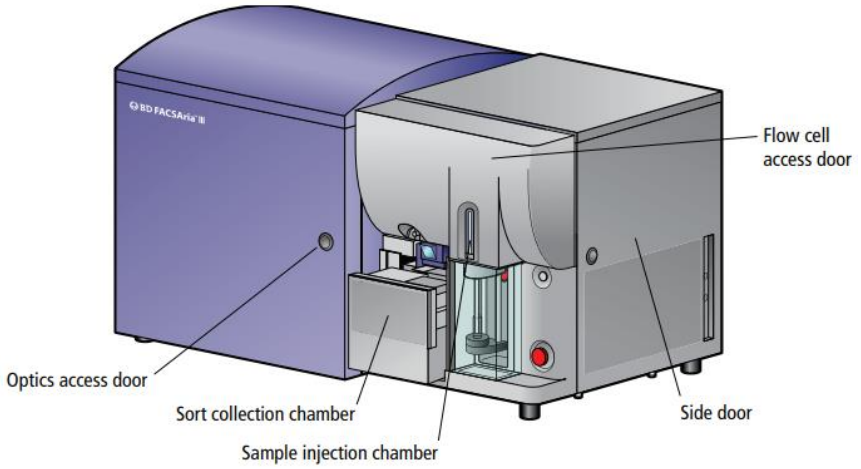
Memorial Sloan Kettering Cancer Center

FACSAria™
Startup, QC & Shutdown Procedure



Flow Cytometry Core Facility

Ver. 2019-12-22

Workflow Overview

Daily Startup Procedure for FACSAria™ Sorters

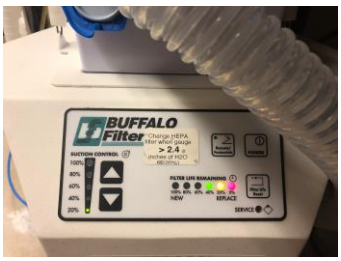
Turn ON Hardware Software ▶ Fluidics & Stream Startup ▶ Cleaning Sort Chamber ▶ Optics QC ▶ Stream/Sort QC Accudrop ▶ Post Sort Cleanup/ Shutdown

1. Turning On Hardware/Software

1. Open Air valve for Aria-2/4. Turn yellow lever on wall until it's parallel with the pipe. **This is not required for Aria-7**.

2. **Aria-2/4:** Turn on Aerosol Management System
Aria-7: Turn on Class 1 BSC by turning Fans on and set AMS fan to 20%

*The AMS should be operating at 20% at all times under normal conditions. If clog occurs ▶ See **Cell Sorter Clog SOP** for further instructions.*



1 **AMS On/Off switch**

2 **100% evacuation button**

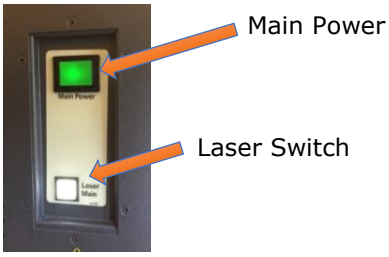
3. Turn ON water bath.
Lauda E100: turn the power switch to on.
Thermo: Press the power button followed by the "enter" button.

Lauda E100 water bath

Thermo water bath

4. Restart computer and Login
Username: Administrator or Admin
Password: BDIS

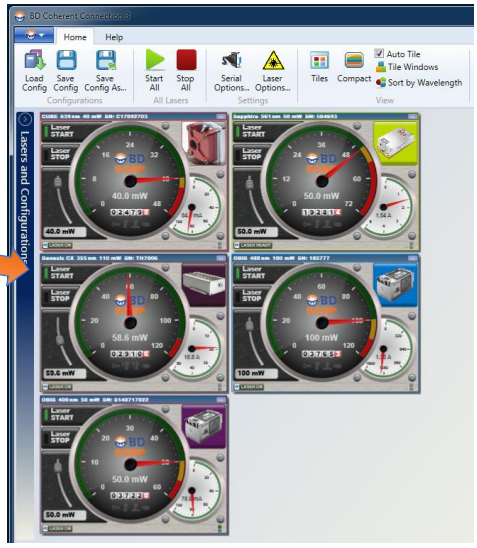
5. Turn ON FACSAria™ sorter.
Once computer is fully on - press the two buttons on the left side of the FACSAria™. Make sure to turn ON the Main Power first and then the Laser Switch button.



6. Launch OBIS/Coherent Connection Software ▸ *Check* ***OBIS/Coherent Connection SOP***

Aria-2: Coherent Connection. UV Laser typically does not start. Set to 5 mW and press start. Once started, set to 60 mW
Aria-4: Does not have Laser connection software.
Aria-7: Does not have Laser connection software.

Coherent Connection on Aria-2. Look for 355 nm laser and power to 60 mW.



7. Launch Diva Software.
Login name: *YourName*
Password: *ID#*

8. Wait for connection.
Choose "Use CST settings" in the dialog box that comes up.

2. Fluidics & Stream Startup

1. Fill sheath tank and empty waste.
Sheath=1xPBS. To prepare, combine 200 mL of 10XPBS with 1800 mL of dH2O.
For Aria-7:
Tue-Fri: No fluidic startup needed. Remove waste container and add bleach to bring it to a final concentration of 10% and leave it to the side. Connect separate empty waste container for startup.
Sat-Mon: Fluidic startup is needed, empty the waste into designated EtOH disposal tanks after startup is completed and connect the empty waste container to the sorter.
For Aria-2/4: at the start of each day empty the waste into the designated EtOH disposal tanks. No bleach should be added. Fluidic startup is done every day.

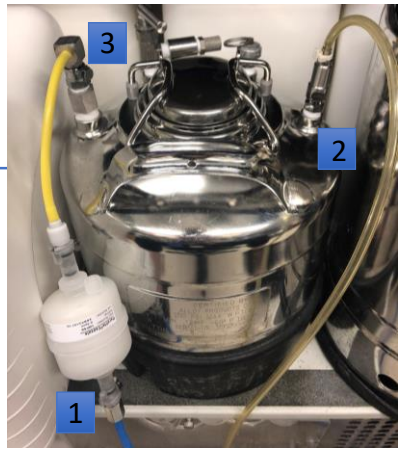
2. Change connections between EtOH tank and Sheath Fluid tank to prepare for sheath startup.

Fluidics setup for EtOH shutdown.
1. Fluidics line hooked up to designated EtOH filter.
2. Pressure line hooked up to male connection on EtOH tank.
3. Release valve down, allowing pressurization of EtOH tank.

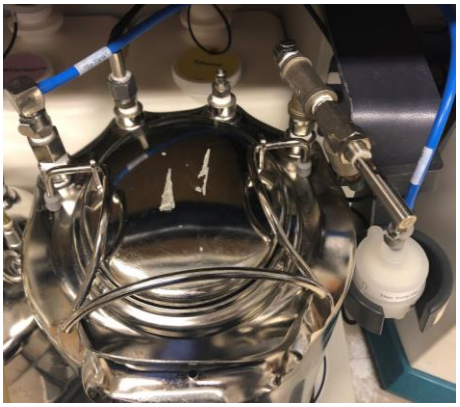
Fluidics setup for Sheath startup.
4. Fluidics line hooked up to designated sheath filter.
5. Pressure line hooked up to male connection on Sheath tank.
6. Release valve down, allowing tank to pressurize with fluidics startup.

*To Refill tank when empty, disconnect pressure line and open release valve to depressurize sheath tank and fill with 1x PBS.

EtOH tank connected

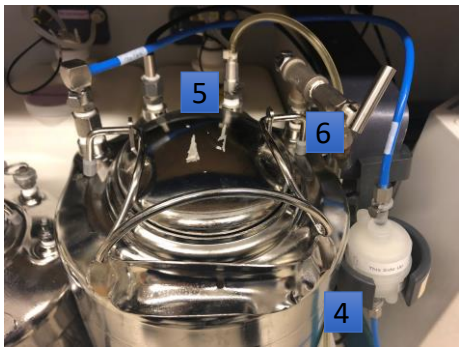


Sheath tank disconnected



EtOH tank disconnected

Sheath tank connected



3. Startup fluidics.
Go to Cytometer ➞ Fluidics Start-up. Follow on screen prompts.

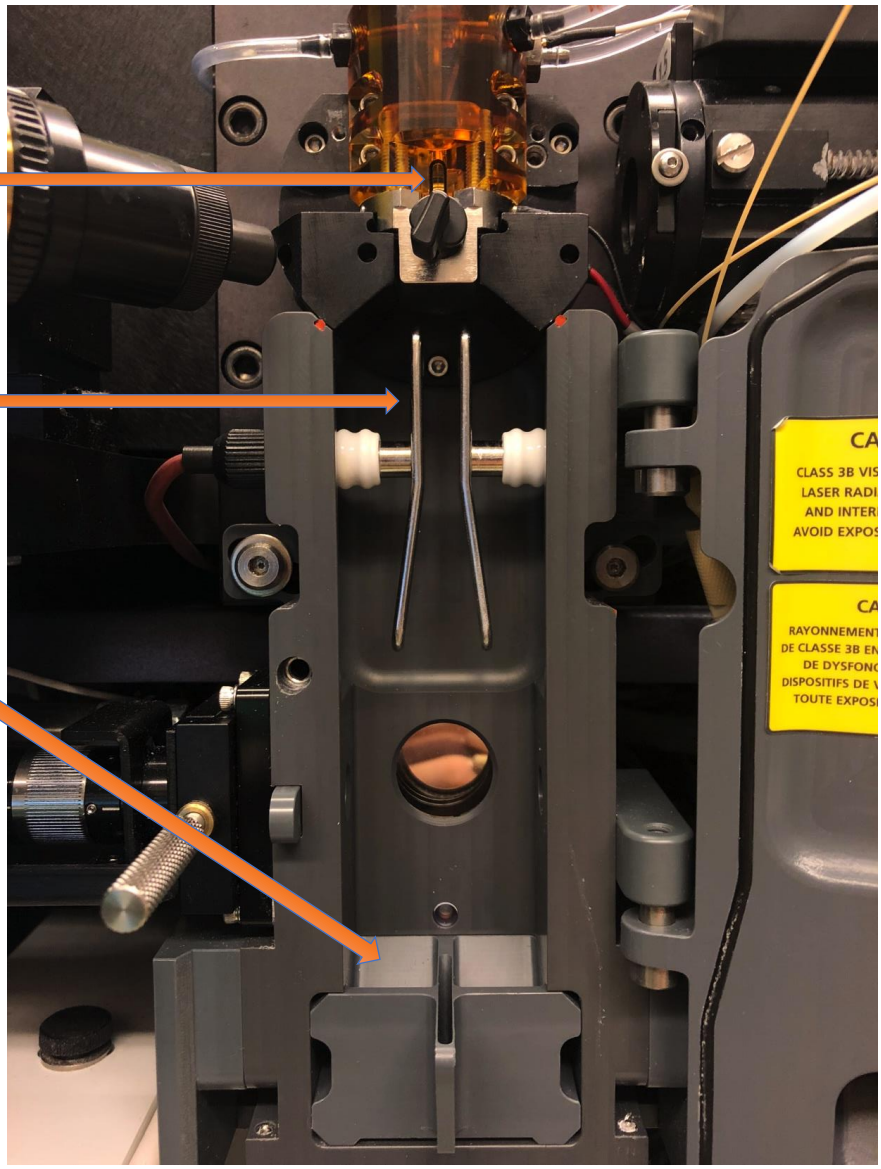
3. Cleaning Sort Chamber

1. Clean interior chamber as well as plates and sort chamber with ethanol.
   Make sure all surfaces are dry.

Flow cell. Check for fluid or salt buildup after removing closed loop nozzle and before inserting sort nozzle.

High voltage deflection plates. Check prior to sort and clean with a moistened kim wipe followed with a dry one to remove any liquid.

Waste drawer. To clean, fill the drawer and the center catch with dH2O to clear out any potential blockage.

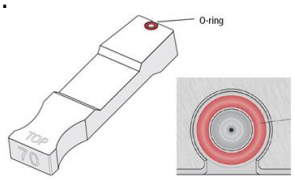


Tip: While the fluidics startup is taking place, clean not only the interior sort chamber but also all work surfaces on the sorter and computer table with 70% ethanol.

2. Sonicate nozzle.
   In dH2O, NO more than 60 s.

3. Insert the nozzle into the flow cell.
   Remove the closed loop nozzle from the flow cell, verify the o-ring is in place, gently dry with a kim wipe and place into nozzle holder. Clean the flow cell with a q-tip if any liquid or buildup is seen. Inspect the nozzle you will be using for your experiment to verify the o-ring is in place and there is no clog, and insert it into the flow cell with the o-ring facing up.

Closed loop nozzle – for cleaning and shutdown

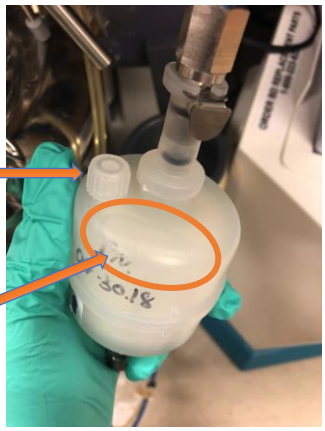


Integrated nozzle – for use during setup and sorting

4 Inspect sheath filter for air bubbles.

If there are any bubbles, bleed the filter after fluidics startup while the tank is pressurized. Be cautious, as the tank is at high pressure and the bleed valve will need to be slightly loosen to ensure it does not come off.

***Note**: Remaining air in the sheath filter can cause instability in the stream during setup and through your run.*



5 Start the stream.

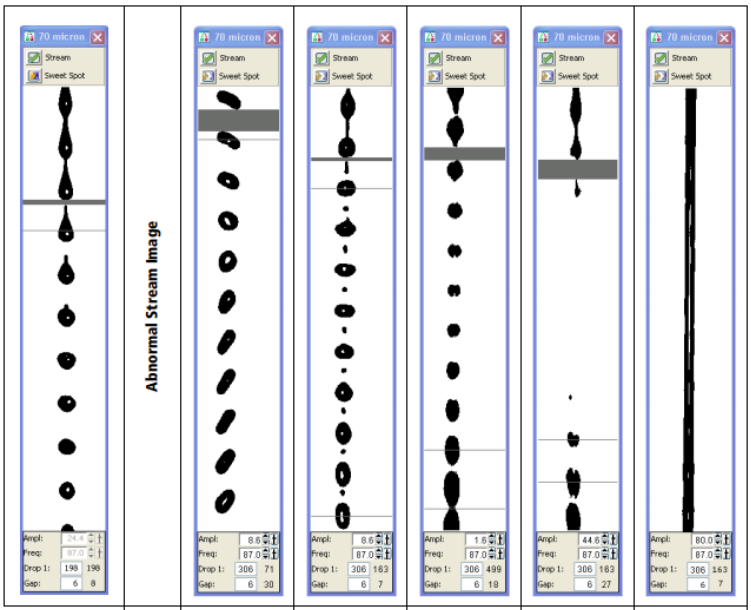
Ensure the correct nozzle size is shown in stream window. Start the stream by clicking the red "X" at the top of the stream window.

Note: If incorrect nozzle size shown ▸ *See **FACSAria™ Nozzle Swap SOP** for further instructions*.

6 Check the stream image and droplet formation.

Confirm with images below to ensure that the droplet formation is normal.

Note: When adjusting the settings for the droplet formation, never change the frequency or the gap that is preset in Diva. These values are optimized for each nozzle on each instrument. Only the amplitude and the drop 1 should need minor modifications to obtain optimal droplet formation.



	Normal stream image	Abnormal Stream Image				
Possible Causes		Nozzle inserted improperly	Nozzle inserted improperly or orifice is off center	Partial clog	Wet or dirty strobe lens	Attenuation is on at wrong pressure
Recommended Solutions		Remove the nozzle and re-insert it.	Remove the nozzle and re-insert it.	Remove the nozzle, clean it, and then re-insert it.	Clean the lens as described in Cleaning the Camera Windows on page 239.	Turn off attenuation in the Side Stream window.

https://www.bdbiosciences.com/documents/BD_FACSAria_II_User_Guide.pdf

7 Turn on the Sweet Spot.

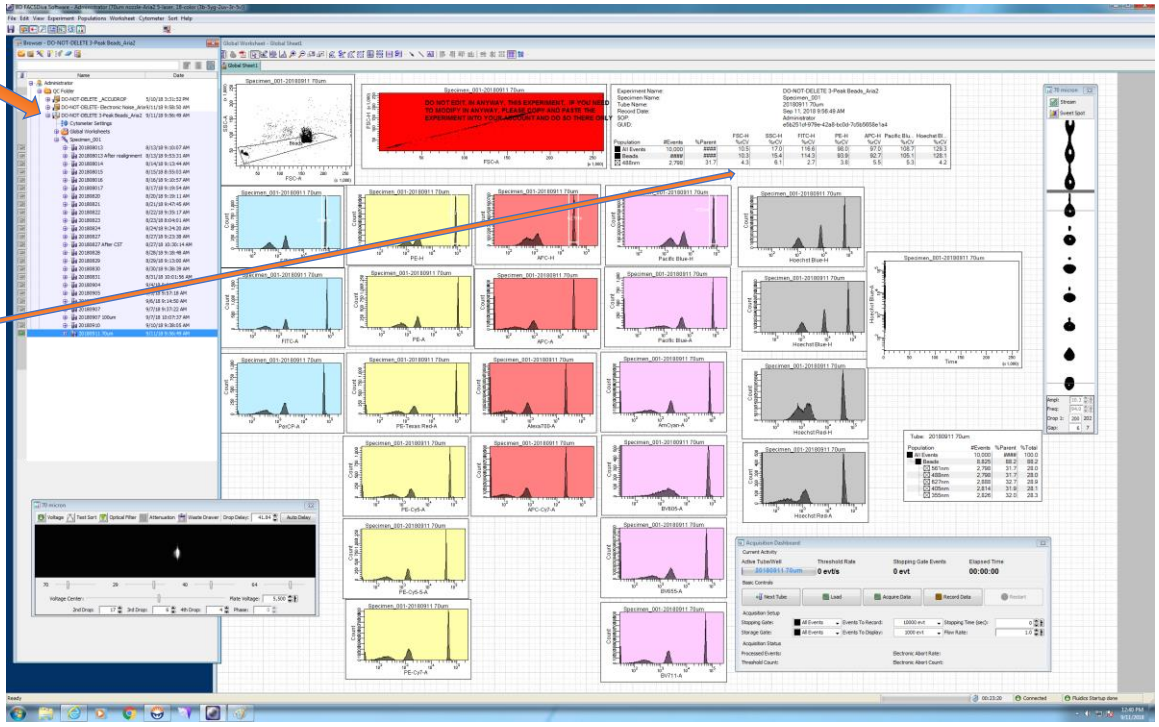
Click the button at the top of the Stream window

4. Optics QC

1. In Diva, go to QC Folder and open the 3-Peak Beads Experiment
2. Create a new tube and name it with the date: yyyymmdd
3. Run the CS&T beads in the 3 peak experiment
   To prepare CS&T beads, add one drop of BD CS&T beads, Cat#655050, into 350uL of 1xPBS. (Refrigerate after use.)
4. Verify that all channels have 3 peaks and brightest peak is falling within target.
   For each laser, the primary fluorescent parameter should have an %rCV below.

Open 3-Peak Beads Experiment.

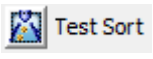
Verify in the statistics box that the bright peak on the primary channel for each laser is below 6.0

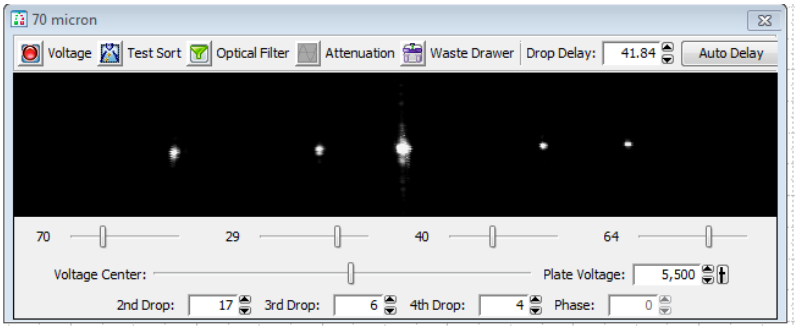


5. Record 5,000 events.

5. Stream Sort QC and Accudrop

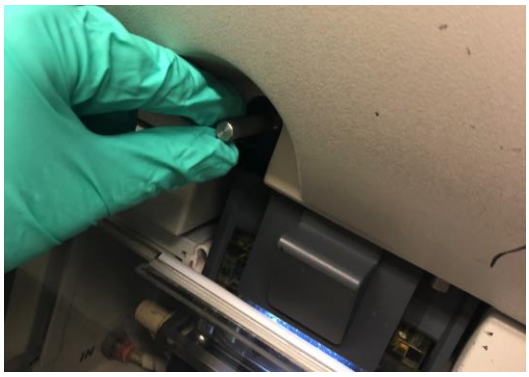
1. Verify quality of charging the stream.
   Turn on voltage 

   and press Test Sort 



2 Optimize Accudrop laser diode.

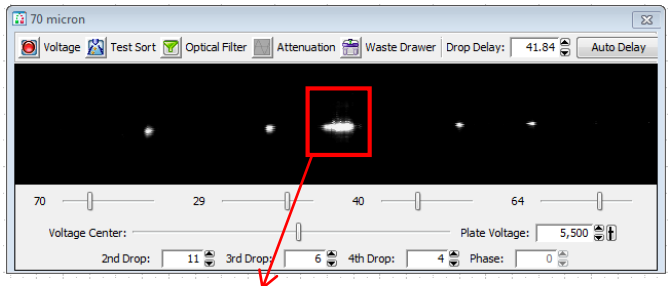
Minimal adjustments should be needed on the laser diode adjustment stage to get the brightest illumination of the streams.



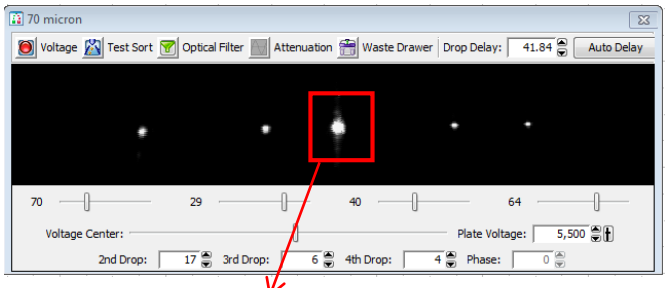
3 Check Fanning.

Adjust 2nd, 3rd and 4th drop in order to minimize any fanning.

Note: Fanning can cause sort impurity.



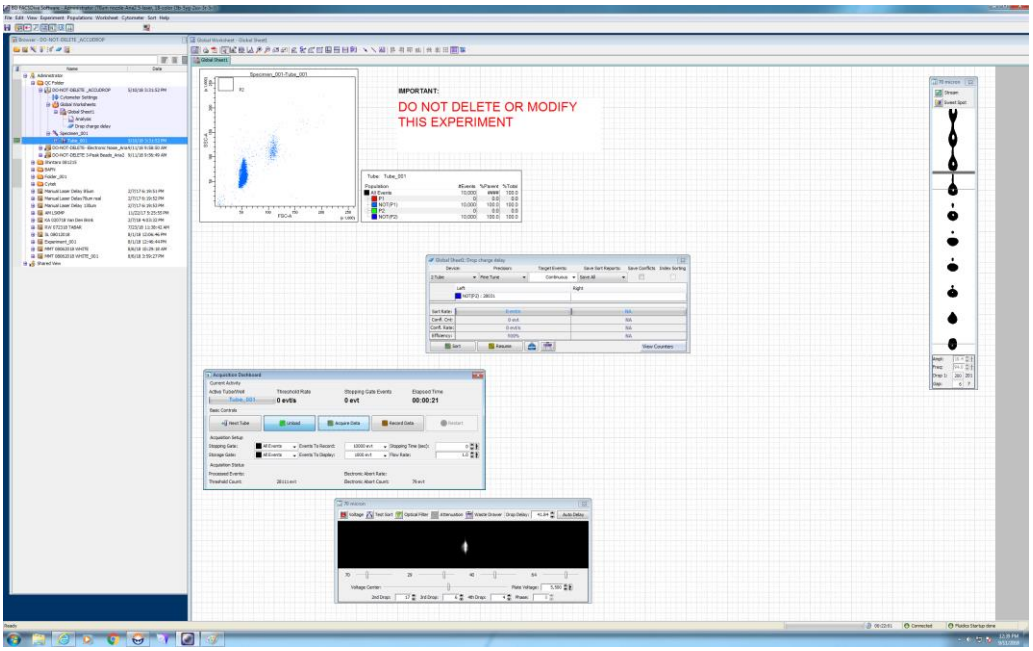
2nd drop is not optimal. Fanning of center stream seen.



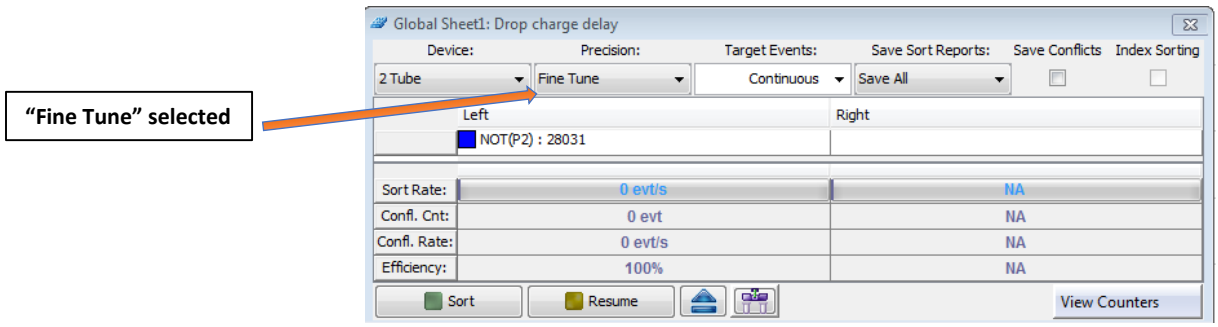
2nd drop optimized. Tight center stream

4 Open Accudrop Experiment in QC Folder ▸ *See **FACSAria™ Drop-Charge Delay SOP***

Select the tube under the specimen and open the Drop Delay Sort Layout under the Global Sheet.



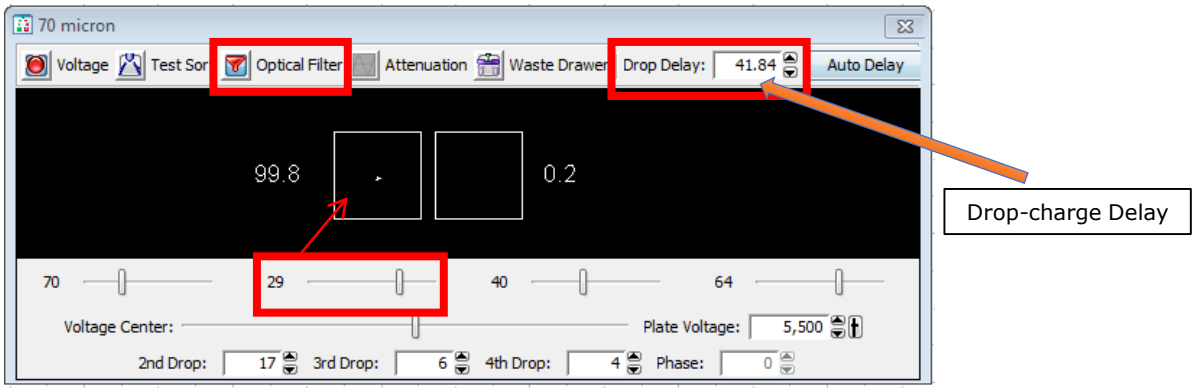
5 Check Sort Precision.
Precision should be at "Fine Tune" in 2 Tube mode.



6 Load & Run Accudrop beads.
To prepare Accudrop beads, put one drop of BD Accudrop beads, Cat#345249, into 700uL of 1xPBS. (Refrigerate after use.) Acquire the beads and run at a flow rate of ~2,000 evts/sec.

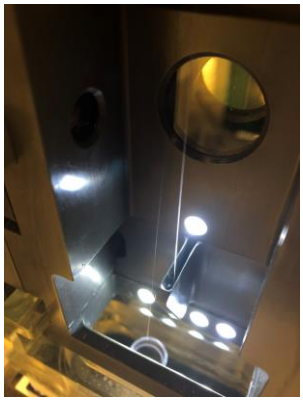
7 Carry out Accudrop sort.
Click "Sort" followed by "Cancel" when the dialog box comes up. Turn on the voltage manually. Confirm that there is no fanning and that the Accudrop laser diode is aligned.

8 Turn on Optical Filter.
Click "Optical Filter" and then adjust the stream deflection so the sort stream falls within the filter.



9 Set Drop-charge Delay ▸ *See **FACSAria™ Drop-Charge Delay SOP** for more detailed instructions*
Adjust Drop-charge Delay value up and down in the Side Stream window until the left stream is ≥98%.
Note: Percentages in center stream and left stream should add to 100%
Increase optimal drop-charge delay 1 whole number. Verify that left stream is ≤2%.
Decrease optimal drop-charge delay 1 whole number. Verify that left stream is ≤ 2%.
TIP: Hold down control and press arrow up or down to change values by one whole integer.

10 Prepare for sort.
Adjust side streams for deflection into appropriate collection vessels and begin your experiment.
For plate sorting ▸ *See **BD FACSAria™ Plate Sorting SOP***.



6. Post Sort Cleanup and Shutdown

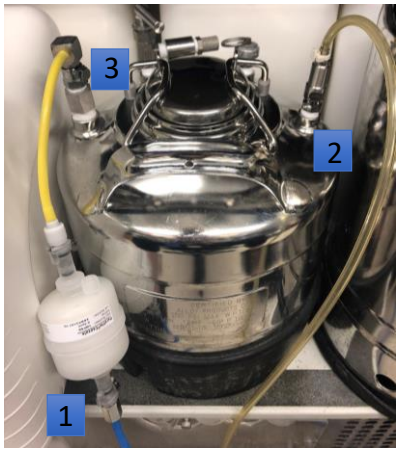
1. Clean the sorter after appointment is complete and check to see if fluidics and/or cytometer shutdown is necessary.

Before 5pm (business weekdays only):
Clean with 2 minutes CLEAN (bleach) at flow rate 11, 2 minutes RINSE (detergent) at flow rate 11, followed by 2 minutes H2O at flow rate 11 and leave instrument on.

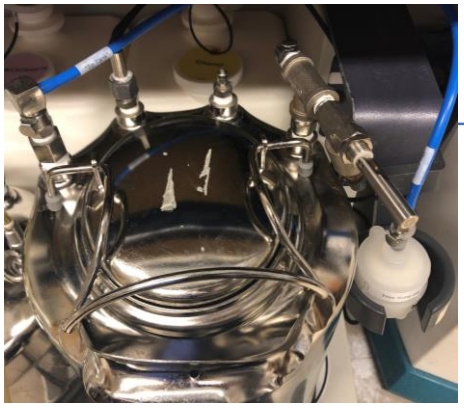
After 5pm (or any time on weekends and holidays):
If someone is booked after your appointment:
Clean with 2 minutes CLEAN (bleach) at flow rate 11, 2 minutes RINSE (detergent) at flow rate 11, followed by 2 minutes H2O at flow rate 11 and leave instrument on.
If no one is booked after your appointment the instrument must be shutdown:
Aria-2/4: Perform Fluidics Shutdown followed by an instrument shutdown (see below 2-4).
Aria-7: Saturday through Thursday: turn stream off, place closed loop nozzle and perform a clean flow cell with water followed by instrument shutdown (see below 4); Friday: perform Fluidics Shutdown followed by an instrument shutdown (see below 2-4)

2. Change connections between Sheath Fluid tank and EtOH Fluid tank to prepare for Fluidic Shutdown.

EtOH tank connected



Sheath tank disconnected



Fluidics setup for EtOH shutdown.

1. Fluidics line hooked up to designated EtOH filter.
2. Pressure line hooked up to male connection on EtOH tank.
3. Release valve down, allowing pressurization of EtOH tank.

*To Refill tank when empty, disconnect pressure line and open release valve to depressurize EtOH tank and fill it with 70% EtOH.

3. Shutdown fluidics.
Go to Cytometer ➞ Fluidics Shutdown. Follow on screen prompts.
Note: When prompted to load a tube of cleaning solution, load a tube of 70% EtOH.

4. Turn off cytometer.
Turn off the main power switch followed by the laser main switch.

5. Turn off AMS or BSC along with the chiller.

Note: Please remember to log out of Diva at the end of your appointment. It is necessary for accurate tracking of usage and billing.